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A NEW COLOR REACTION FOR CAFFEINE

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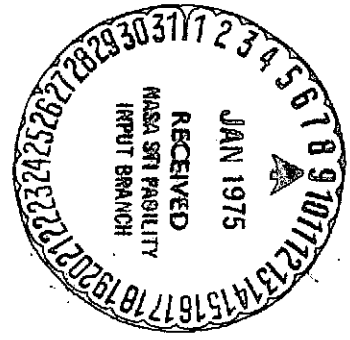

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A NEW COLOR REACTION FOR CAFFEINE

H. Wachsmuth and L. VanKoeckhoven

The qualitative and quantitative reactions of caffeine are /79* not large in number. The classical qualitative color reactions are those of HNO_3-NH_4OH and phosphotungstic reagents. The quantitative analysis procedures are based on "Kjeldahlization," on gravimetric quantitative analysis, on precipitation by heteropolyacids or iodized reagents, on ultraviolet spectrophotometry, and on perchloric quantitative analysis in organic solutions. Various reactions and methods of quantitative analysis have also been proposed in the last two years, but with some exceptions, they involve primarily procedures derived from older, slightly modified techniques.

The reaction that we propose is negative with closely related purine-like bases such as theobromine and theophylline (as well as euphylline). Furthermore, the purines and pyrimidines enumerated below furnish equally a negative reaction: adenine, guanine, hypoxanthine, xanthine, uric acid, thymine, and uracil.

Finally, among approximately fifty alkaloids and basic substances, only caffeine provided a positive reaction on the chromatogram:

*Numbers in the margin indicate the pagination of the original foreign text.

acedicon	eumydrin
aconitine nitr.	heroin HCl
alipine HCl	homatropine HBr
atropine sulf.	hydrastinine HCl
benzedrine sulf.	hydrastine
betaine HCl	khellin
brucine	mephenon
chelidonine	morphine HCl
choline HCl	morpholine
cicutine HBr	narceine HCl
cocaine HCl	narcotine HCl
codeine phosph.	nicotine
colchicine	novocaine HCl
dicodid	pantocaine
dilaudid	papaverine HCl
dionine	pervitine HCl
dolantin	pilocarpine HCl
emetine HCl	privine
ephedrine HCl	quinidine sulf.
ethanolamine (mono-)	quinine HCl
ethanolamine (di-)	scopolamine HBr
ethanolamine (tri-)	sparteine sulfate
eucodal	strychnine nitr.
	sympathol

(all negative)

The reaction thus appeared to be specific and it lent /80 itself to quantitative determination. The coloring is still clearly perceptible in a dilution of 1:2,000,000 under a layer 10 mm thick. Spectroscopically, a band of absorption is obtained at the red limit with maximum at about 610 mμ.

Technique

Caffeine dissolved in an alkaline solution and mixed with several drops of acetylacetone 2,4 pentanedione is submitted to heating. After recooling, a hydrochloric solution of p-dimethylaminobenzaldehyde is added. With the addition of water, a good, blue color spreads throughout. It is, however, well advised to perform this reaction in well defined conditions. The nature and quality of alkali, the temperature and duration, the quantity of acetylacetone, the concentration of dimethyl-aminobenzaldehyde and of hydrochloric acid condition the development of the reaction.*

Quantitative Analysis

Reagents: A. Solution of acetylacetone
0.5 ml acetylacetone + 5 ml NaOH
(prepare by improvisation)

B. Solution of p-dimethylaminobenzaldehyde
0.100 g p-dimethylaminobenzaldehyde +
2 ml conc. HCl

The caffeine is dissolved in 0.25 ml of acetylacetone solution (A). The tube is stoppered and kept at 80° C for seven minutes. After recooling, 0.5 ml of reagent (B) is added and the solution is again heated for seven minutes at 80°. After cooling again, 10 ml of water is added and the wavelength is immediately measured at 615 mμ.

*The blue color develops with the addition of water. By replacing water with a solution buffered by phosphates (pH = 7.2), the intensity of the coloration is slightly greater.

Sample	D.O. (10 mm)	D.O./100 µg
100 µg caffeine	0.305	0.305
200	0.605	0.303
300	0.912	0.304
400	1.220	0.305
100 µg caffeine	0.300	0.300
200	0.604	0.302
300	0.910	0.303
400	1.210	0.302

Sample	D.O. (10 mm)
200 µg caffeine	0.605
200 µg caffeine + 500 µg theobromine	0.605
200 µg caffeine + 500 µg theophylline	0.605
400 µg caffeine	1.200
400 µg caffeine + 500 µg theobromine	1.200
400 µg caffeine + 500 µg theophylline	1.200

The quantitative analysis of caffeine in the presence of /81
theophylline and theobromine thus furnishes precise results. Because of this fact, the method could be applied to the determination of caffeine in coffee (even decaffeinated), tea, cocoa, Paraguay tea (mate), and others.

Conclusion

We propose a new color reaction of caffeine. The reaction is very sensitive, appears specific, and lends itself to quantitative determination, even in the presence of theobromine and theophylline, or of other purine-like bases and pyrimidinic bases. Alkaloids do not interfere.

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